

growing ability based on protoporphyrinogen oxidase activity, said vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell;

E₁ cont'd
(2) culturing said transformant expressing said protoporphyrinogen oxidase DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indicative of an inhibition of protoporphyrinogen oxidase activity by said test compound.

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~~23~~. (Twice Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen oxidase activity,

said vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell, and a terminator functional in the host cell;

G₁ cont'd
(2) culturing said transformant expressing said protoporphyrinogen oxidase DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indicative of an inhibition of protoporphyrinogen oxidase activity by said test compound.

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30. (Twice Amended) A method for evaluating the ability of a compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen oxidase activity,

E₁ Cont'd
said vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell, wherein said promoter is inducible, and a second vector comprising a second DNA fragment which is a DNA capable of inducing the promoter of the first DNA fragment, and a promoter, wherein said promoter is not induced by the second DNA fragment but is functional in the host cell, are operatively linked;

(2) culturing said transformant expressing said protoporphyrinogen oxidase DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative system, wherein an inhibition of the growth rate is indicative of an inhibition of protoporphyrinogen oxidase activity by said test compound.

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~~31~~. (Twice Amended) A method for evaluating the ability of a

compound to inhibit protoporphyrinogen oxidase activity, comprising the steps of:

E₁ cont'd
(1) transforming with a vector a host cell deficient in growing ability based on protoporphyrinogen oxidase activity, said vector comprising a DNA fragment coding for enzyme protoporphyrinogen oxidase which is capable of oxidizing protoporphyrinogen into protoporphyrin and which confers growth ability, wherein said DNA fragment is operably linked to a promoter functional in said host cell, and a terminator functional in the host cell, wherein said promoter is inducible, and a second vector comprising a second DNA fragment in which a DNA being capable of inducing the promoter of the first DNA fragment, a promoter, wherein said promoter is not induced by the DNA fragment but is functional in the host cell, and a terminator functionable in the host cell are operatively linked;

(2) culturing said transformant expressing said protoporphyrinogen oxidase DNA in a medium containing substantially no protoheme compounds, wherein in a first comparative system there is a presence of a test compound to measure a growth rate of the transformant and in a second comparative system there is an absence of said test compound; and

(3) determining the ability of the test compound to inhibit the protoporphyrinogen oxidase activity by comparing the growth rates of the first comparative system to the second comparative

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E₁ control
system, wherein an inhibition of the growth rate is indicative of an inhibition of protoporphyrinogen oxidase activity by said test compound.
